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X/Autosome Translocation in Three Generations Ascertained Through an Infant With Trisomy 16p Due to Failure of Spreading of X-Inactivation

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We report on a reciprocal translocation t(X;16)(q28;p12) detected in a newborn girl with clinical manifestations of partial trisomy 16p. A balanced translocation was found in the mother and in the maternal grandmother. Replication studies on lymphocytes and fibroblasts showed nonrandom X-inactivation in both the patient and her mother. In the mother, the derivative X (der(X)) was active, whereas the normal X was late replicating. In contrast, in the patient the der(X) was late replicating, and there was no spreading of X-inactivation onto the autosomal segment, thus giving an explanation for the full clinical picture of partial trisomy 16p. © 1996 Wiley-Liss, Inc.

KEY WORDS: trisomy 16p, familial X/autosome translocation, X-inactivation, late replication, failure of spreading

INTRODUCTION

Complete trisomy 16 is not compatible with postnatal life and causes about 15% of all spontaneous early abortions [Schinzel, 1984]. Since 1977, at least 25 cases of trisomy 16p have been reported from 16 families, mostly due to a maternal reciprocal translocation with another autosome [Roberts and Duckett, 1978; Dallapiccola et al., 1979; Leschot et al., 1979; Mori et al., 1987; Jalal et al., 1989; Bofinger et al., 1991; Léonard et al., 1992; O'Connor and Higgins, 1992; Brandt et al., 1994]. In this report we describe a case resulting from a translocation to the long arm of the X chromosome.

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CLINICAL REPORT

Our patient (Fig. 1) is the daughter of a 19-year-old Turkish primagravida, and her 25-year-old husband. Both parents are healthy and non-consanguineous. Apart from 4 abortions of the maternal grandmother, the family history was unremarkable. The pregnancy was uneventful, and birth occurred spontaneously at 38 weeks of gestation. Birthweight was 2,430 g. Apgar scores were −/10/10. The child showed evident minor anomalies. For comparison, the frequency of the main findings seen in 19 cases compiled by Léonard et al. [1992] are given in brackets: narrow palpebral fissures (No. of cases 13/No. of informative cases 13), sparse eyebrows (9/10), apparently low-set posteriorly angulated but otherwise normal ears (12/15), prominent glabella (12/12), prominent supraorbital region, broad nasal bridge (13/14), rounded nasal tip (13/13), anteverted nares (13/13), prominent maxilla (9/12), microretrognathia (14/16), inverted upper lip (9/12), median cleft palate (14/15), clasped thumbs (6/9), overlapping fingers II and V (13/19), rocker bottom foot with vertical talus on the right, and neonatal pes calcaneus on the left, with limited abduction of the hips. The internal organs were considered normal on ultrasonography.

On reexamination 3 months later, severe growth retardation (11/11) was evident (weight 4,170 g (10th–25th centile), length 45.5 cm (below 3rd centile), and head circumference (OFC) 32.5 cm (below 3rd centile) (9/13)). The child was hypertonic (5/8) and presented an opisthotonus with asymmetric posture. Eye fixation and head control was not possible. In addition, an atrial septal defect type II with left-right shunt (cardiac anomaly 8/15), a paracardial diaphragmatic eventration (2.5 × 3 cm) on the right side, and subluxation of the hips were diagnosed.

Cytogenetic Studies

Chromosome analysis of the patient's lymphocytes using G banding (trypsin technique, GTG) showed a structurally altered X chromosome with an elongated long arm (Fig. 2a). Subsequently, a balanced X/autosome (X/A) translocation could be demonstrated in the mother's lymphocytes (Fig. 2b). Because of the very ter-

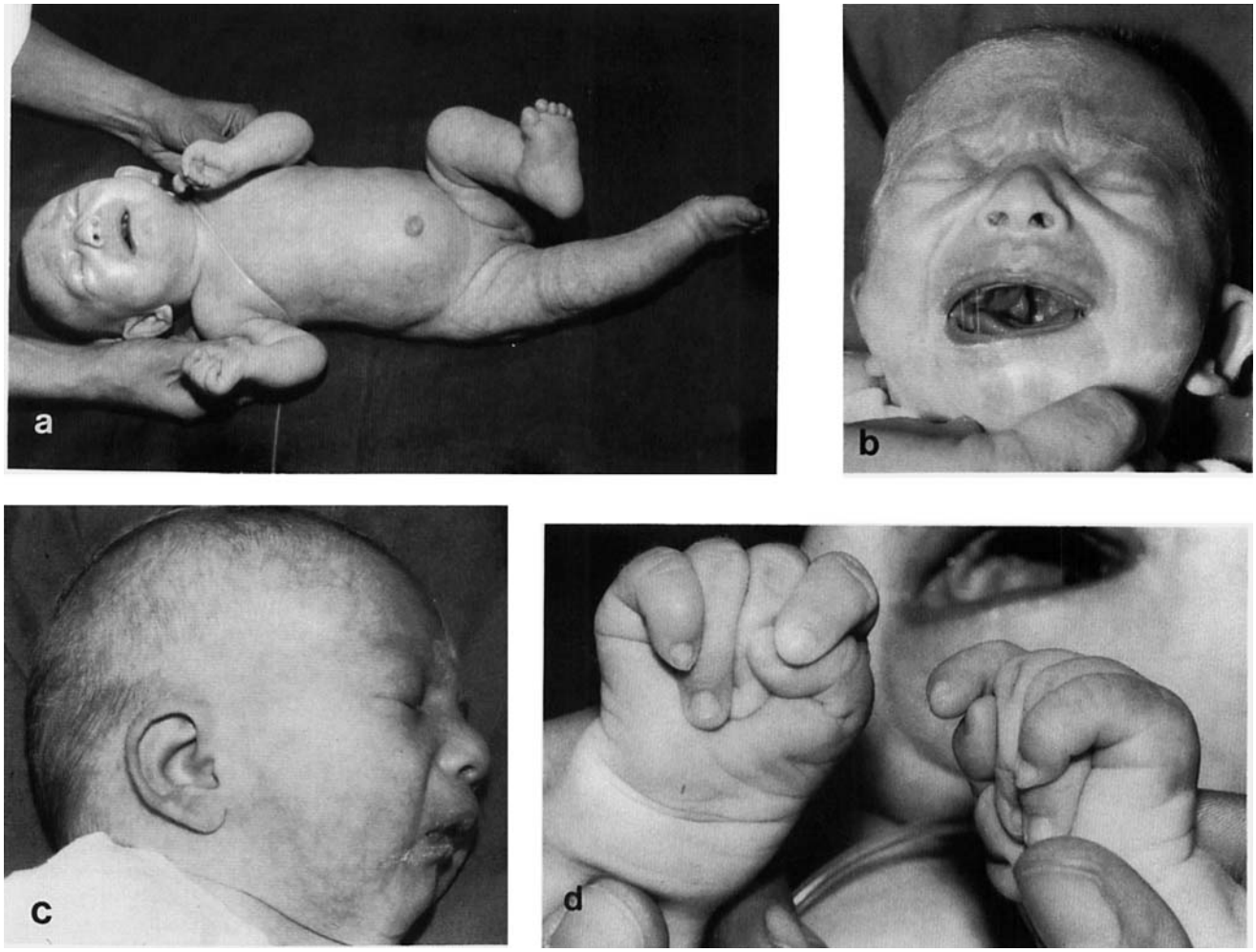

Fig. 1. **a–d:** Our patient at age 15 days.

minal breakpoint on Xq, fluorescence in situ hybridization (FISH) with an X-specific library (AGS, Heidelberg) was performed to demonstrate the terminal Xq segment on the derivative chromosome 16 (Fig. 3). Thus, the maternal karyotype was determined as 46,X,t(X;16)(q28;p12). Consequently, the patient's chromosomal imbalance could be interpreted as partial trisomy 16p (karyotype: 46,X,der(X)t(X;16)(q28;p12)mat). The translocation was inherited from the maternal grandmother.

Replication studies were carried out on the patient's and her mother's lymphocytes and fibroblasts using the RBG technique [Epplen et al., 1975]. These showed nonrandom X-inactivation in both persons: In the mother (Fig. 4a) the der(X) chromosome was early, and the normal X was late replicating in all metaphases analyzed from both lymphocytes (n = 60) and fibroblasts (n = 50). In contrast, the patient's der(X) was late replicating in all metaphases studied (lymphocytes: n = 15; fibroblasts: n = 35) showing early replication only of the translocated autosomal segment (Fig. 4b). In each metaphase analyzed, there was a clear-cut change of replication timing at the translocation breakpoint. No spreading of X-inactivation onto the autosomal segment of the late replicating der(X) could be detected.

DISCUSSION

We report on an X/A translocation in 3 generations ascertained through an unbalanced offspring. Until now, at least 25 cases of trisomy 16p have been reported [Léonard et al., 1992; O'Connor and Higgins, 1992; Brandt et al., 1994], mostly due to a maternal translocation with different autosomes involved (chromosomes 7, 9, 10, 12, 13, 14, 15, and 21). Despite differences in the extent of the duplicated segment, the cases are clinically rather uniform. Apart from the dislocated hips and diaphragmatic eventration, our patient presented no striking differences from the reported cases. Besides further support for the delineation of a trisomy 16p phenotype, our observation also provides insight into cell selection with regard to X-inactivation patterns in a case of an X/A translocation.

Though our understanding of X-inactivation has increased steadily [reviews by Lyon, 1992; Riggs and Pfeifer, 1992; Migeon, 1994; Distèche, 1995] many

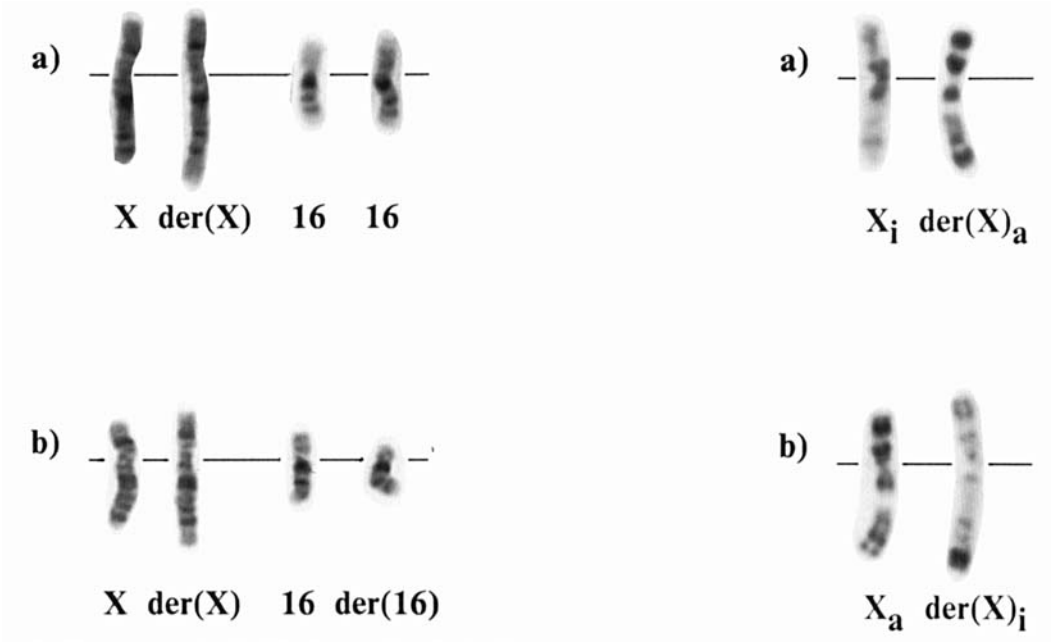

Fig. 2. **a,b:** Chromosomes involved in the translocation in the patient (**a**) and her mother (**b**) after GTG-banding. The breakpoints are indicated by arrows.

Fig. 4. **a,b:** RBG-replication-banding. Note inactivation of the normal X in the mother (**a**), and failure of spreading of late replication onto the autosomal segment of the patient's der(X) (**b**).

questions have remained open. It is accepted that late replication is a cytogenetic parameter indicating X chromosome inactivation [Taylor, 1960; Couturier et al., 1979; Mohandas et al., 1982]. From studies on balanced and unbalanced X/A translocations, we have come to understand nonrandom X-inactivation as the result of selection against maximum chromosomal imbalance in a cell population in which random inactivation has occurred [Gartler and Andina, 1976] rather than of selective inactivation of a certain X chromosome. Our observations are compatible with this view.

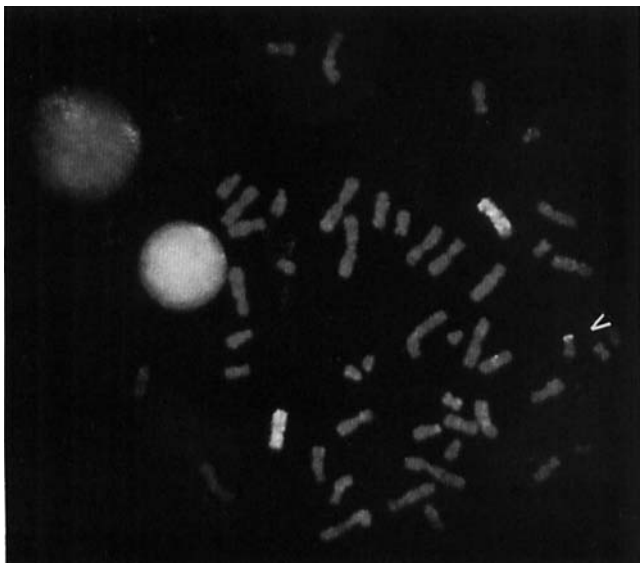

Fig. 3. Metaphase spread from the mother after FISH with an X specific library. The reciprocal nature of the translocation is proven by demonstration of an X specific signal on the der(16) chromosome (see arrow).

The observed pattern of nonrandom inactivation of the mother's lymphocytes and fibroblasts is in agreement with her normal phenotype. Inactivation of the normal X is the only way to achieve a functionally balanced situation in cells with a cytogenetically balanced X/A translocation. Since inactivation of distal Xq28 attached to the der(16) is not possible, inactivation of the der(X) instead of her normal X would lead to functional disomy of this segment. Furthermore, functional monosomy of the autosomal segment translocated to an inactivated der(X) would only be prevented by failure of spreading. At least in some carriers of balanced X/A translocations with phenotypic abnormalities, the existence of cells with a late replicating der(X) in addition to cells with a late replicating normal X has been reported [Schmidt and Du Sart, 1992], indicating primary random X-inactivation and giving a causal explanation of their phenotypes. However, functional disomy of Xq28 cannot be expected in a normal mother. Furthermore, monosomy of almost the entire 16p arm has to date not been reported and thus seems to represent a lethal condition.

Our patient is an example of maximum autosomal imbalance in an unbalanced X/A translocation due to failure of spreading of inactivation onto the autosomal segment. Late replication of the der(X) was observed exclusively. Even this situation can be regarded as the result of selection after originally random X-inactivation. Inactivation of her normal X would have led to deleterious functional nullisomy of Xq28. According to our replication studies on lymphocytes and fibroblasts, there is no hint of inactivation of the duplicated autosomal segment even though it is translocated to the inactive X

chromosome. This finding is in agreement with her phenotype, identical with the clinical manifestations in patients with "pure autosomal" partial trisomy 16p.

To date the phenomenon of spreading of X-inactivation is only poorly understood, especially in X/A translocations, where autosomal chromatin has become late replicating. In balanced reciprocal X/A translocations, functional imbalance is avoided by inactivation of the normal X chromosome. In the case of an inactive der(X) bearing the inactivation center, monosomy for the translocated autosomal segment can still be prevented by failure of spreading. In unbalanced reciprocal X/A translocations, a continuous spectrum of spreading of late replication onto the autosomal segment has been observed, varying between almost complete [Leisti et al., 1975], or partial [Zuffardi et al., 1977; Allderdice et al., 1978; Couturier et al., 1979; Taysi et al., 1982; Rivera et al., 1984; Schanz and Steinbach, 1986], or missing late replication, respectively [Cohen et al., 1983; Camargo and Cervenka, 1984; Keitges and Palmer, 1986].

In our patient, inactivation is apparently restricted to the X chromosomal segment of the translocated X chromosome. Such "selective" spreading is often observed in X/A translocations with terminal breakpoints [Palmer et al., 1980; cf. Table III of Elejalde and de Elejalde, 1983; Keitges and Palmer, 1986], a finding suggestive of a hypothetical terminal boundary of spreading on both arms of the X chromosome. A breakpoint in the pseudoautosomal region, which escapes X-inactivation, would explain failure of spreading onto the autosome at least in a proportion of cases with breakpoints at terminal Xp. Taking into account that precise comparison of cytogenetically defined translocation breakpoints is not possible, the findings of late replication in autosomal segments distal to Xq28 in unbalanced as well as in balanced reciprocal X/A translocations [Jenkins et al., 1974; Keitges and Palmer, 1986] allow no demonstration of a terminal boundary of spreading in Xq. Schanz and Steinbach [1986] reported on a case of an unbalanced X/A translocation, where the degree of spreading onto the translocated autosome varied between the cells, and they showed that this variation of spreading within the autosomal segment is not maintained in a clonal manner. They suggested that variable spreading might rather result from ineffective maintenance of inactivation on the autosome. Since maintenance of spreading requires a cis-acting mechanism, its fidelity could decrease with the distance between the inactivation center and the autosomal segment. To date investigations on spreading in X/A translocations have been restricted to replication studies, and it is still unknown if the observed differential fidelity of spreading between X chromosomal versus autosomal segments depends on the origin of chromatin involved. Variable spreading might depend on inherent properties of the chromatin at the translocation breakpoint or merely be the result of failing maintenance of late replication on a chromosome once completely inactivated. Though not a sufficient condition, methylation of CpG sites plays an important role in X-inactivation, thus giving a molecular explanation of clonal maintenance of late replication [Riggs and Pfeifer, 1992; Migeon, 1994]. "Hypomethylation" of an inactive X chromosome, as inducible by incorporation of the thymine analogue 5-azacytosine, may not only lead to reactivation of silenced genes [Mohandas et al., 1981], but may also shift replication timing from "late" to "early" [Jablonka et al., 1985; Schmidt et al., 1985]. The fidelity of a maintenance system based on methylated CpG sites, though not perfect [Riggs and Pfeifer, 1992], might be greater on X chromosomal than on autosomal chromatin.

Our observation of absent spreading of inactivation onto the autosomal segment of an X/A translocation chromosome is only a finding of cytological resolution. To test the hypothesis of a differential chromatin effect for X chromosome and autosomes with respect to spreading, further investigations are necessary to better characterize the X/autosomal boundary with regard to methylation.

Karyotype-phenotype correlation in cases of an X/A translocation will remain imprecise, at least as long as the distribution of somatic mosaicism with respect to X-inactivation, the criteria for selection of the resulting cell line(s), and the mechanisms of spreading and maintenance of inactivation are not understood.

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